demonstrate the importance of both the SecA N-domain as well as its C-domain in stabilizing the interaction in vivo.

286-Pos

Increasing Chloride Conductance Through the SecY Complex By Mutagenesis or Trivalent Cations

Huan Bao, Kush Dalal, Franck Duong.
University of British Columbia, Vancouver, BC, Canada.

The SecY complex or channel is responsible for translocation of proteins across the bacterial inner membrane. The complex maintains a seal for small molecules by means of a plug domain and a hydrophobic pore, consisting of six isoleucine residues arranged in a ring. When these pore residues are mutated into asparagine, a specific conductance for small monovalent anions, like chloride, is observed. Here, we show that an enhanced chloride conductance is also observed when bulky phenylalanine residues are introduced into the pore ring. The increased conductance is accompanied by an increase in protein translocation. Chloride conductance was also observed upon addition of trivalent aluminum cations, which are suspected of binding to negatively charged residues near the lateral gate of SecY.

287-Pos

Stability of Vesicles With Incorporated Aquaporin Z Under Various Physiochemical Conditions

Jinsoo Yi[1], Jens Nording[1], Julie Valbjorn[1], Chandrasmitha Bhatt[1], Per Brandt Rasmussen[1], Anitta Krogh Jensen[1], Carlo David Montemagno[2], Jørgen Steen-Pedersen[1].
[1]Danfoss AquaZ A/S, Nordborg, Denmark, [2]University of Cincinnati, Cincinnati, OH, USA.

Stability of Vesicles with Incorporated Aquaporin Z Under Various Physiochemical Conditions

Aquaporins are integral membrane proteins that can transport large amounts of water molecules across the cell membrane by facilitated transport. One aquaporin can transport over 3 billion water molecules per second. Among many different types of aquaporins, we are using *E. coli* Aquaporin Z that is known as a highly specific water channel. This prokaryotic origin aquaporin is easy to modify genetically and it is possible to produce it in large amounts. By means of nanobiotechnology we attempt to develop aquaporin-incorporated membranes that can act as energy efficient water filtration membranes. To fabricate the aquaporin incorporated filters, it is important to characterize the aquaporin endurance against various chemical and physical conditions, in order to know what type of nanofabrication technologies can be used in the final membrane production. In our membrane concept, aquaporin incorporated vesicles are the main building blocks because thermodynamically vesicles are stable and aquaporin retains its functionality when incorporated into vesicles. In this research, we have tested aquaporin-incorporated vesicle functionality with respect to various physiochemical influences such as pH, high temperature, long time UV exposure, high pressure, oxidative stress and several solvents at various concentrations. Subsequently, we have characterized the effect of the applied physiochemical conditions by stopped flow light scatter (SFLS) to check if the water permeability of the vesicles was changed or killed by the various treatments.

288-Pos

Atomistic Model For the Outward-Facing State of Lactose Permease and Quantification of Ligand Binding

Pushkar Y. Pendse, Jeffery B. Klauda.
University of Maryland, College Park, College Park, MD, USA.

The Major Facilitator Superfamily (MFS) is an important class of membrane transporters whose members are found in unicellular organism to complex systems like the human central nervous system. Lactose Permease (LacY) of E. coli, an MFS protein that transports various sugar molecules across plasma membrane, has been studied as a prototype of secondary transport proteins. Though the structure of LacY in the cytoplasmic open state (inward-facing) has been determined, the atomic-level details of the periplasmic open structure (outward-facing) are unknown. Using a two-step hybrid simulation approach that involves Self-Guided Langevin Dynamics (SGLD) simulations with an implicit membrane followed by molecular dynamics simulations with an explicit membrane, we have obtained an opening of LacY on the periplasmic side that is consistent with the Double Electron-Electron Resonance (DEER) experiments (Smirnova et al., PNAS, 2007). The inward-facing state is stabilized by several helix-helix hydrogen bonds involving side chains of the residues N245, S41, E374, K42 and Q242 and mutations in these residues may destabilize these interactions and help crystallize the outward-facing state. We believe that this hybrid simulation approach can be extended to determine the unknown structures and mechanisms of other secondary active transporters. In addition, sugar binding to LacY has been investigated and our simulations support the alternating access model of sugar transport (same binding site accessible from either side of the membrane). More extensive studies on binding involved alchemical free energy perturbation calculations on αβ-(Galp)2 and ββ-(Galp)2 to better understand the anomeric binding effect. Our methods are tested by comparing the computed binding free energy values for NPG (p-nitrophenyl α-D-galactopyranoside) with the experimental values (Nie et al., JBC, 2006). This work will aid in understanding the effect of substrate structure and affinity to LacY.

289-Pos

Single Molecule Studies of *E-ColiF$_1$F$_o$ ATP Synthase* in Lipid Bilayers

Wei M. Ho, Richard M. Berry.
University of Oxford, Oxford, United Kingdom.

While rotation of *F_1 ATPSynthase* has been well characterised at the single molecule level over the past twelve years, direct observation of coupled rotation of *F_1F_o ATP Synthase* in energised lipid bilayers is still fraught with difficulties. One of the biggest challenges is the creation and preservation of a stable and energisable lipid bilayer, so that the proteins can be inserted and remain functional in its native environment. Such a setup must also be coupled with a high resolution microscopy technique, in order to allow direct single molecule observations. In this study, we report the use of the *droplet-on-hydrated-support bilayers* (*DHBs*) technique, by Heron et. al. , to observe both fluorescence labelled and gold labelled *EColiF$_1$F$_o$ATPase*, which have been inserted into the lipid bilayer. This was done using *Total Internal Reflection Microsopy* (*TIRF*) and a novel Dark Field Microscopy setup. The 2-dimensional diffusional constant of the protein ensemble in the lipid bilayer was characterised by tracking the position of the proteins over time. Furthermore, we have also attached the F_o portion of the protein to a functionalised substrate sitting below the bilayer and thereby inhibiting its movement. The F_1 portion is, in turn, labelled with gold beads and free to rotate above the bilayer. This configuration would allow the F_o to behave as the stator while the F_1 as the rotor, so that coupled rotation can be observed. The next part of this on going work would thus be to provide either ATP or a proton motive force to drive the rotation of the protein in hydrolysis and synthesis mode. We hope to report these findings in the coming conference.

290-Pos

Exploring Conformational Changes in the RbsABC Transporter Using EPR Spin Labeling

Michael J. Simon[1], Matthew C. Clifton[2], Mark A. Hermodson[1], Huide Zhang[1], Cynthia V. Stauffacher[1].
[1]Purdue, West Lafayette, IN, USA, [2]Fred Hutchinson Cancer Center, Seattle, WA, USA.

ATP Binding Cassette (ABC) transporters are transmembrane transporters that use the energy released by ATP hydrolysis to transport a wide array of substrates. They are found in all kingdoms of life, and are complicit in various genetic conditions, such as cystic fibrosis, macular degeneration, and multi-drug resistance. The E. coli ribose transporter (RbsABC) is a multisubunit ABC transporter complex with a periplasmic ribose binding domain, a transmembrane domain dimer, and a cytoplasmic nucleotide binding domain. The ribose transport complex has been shown to assemble and disassemble into distinct combinations of the subunits based on the presence of cofactors (ATP and analogues, ADP, orthovanadate, and magnesium), suggesting a series of steps for how the subunits associate and subsequently transport ribose.

To further explore the conformation of the subunits in response to these different sets of cofactors, cysteine mutations were introduced to allow the addition of EPR spin labels. These labeled mutants will be used to determine whether subunits are bound. Additionally, double mutants will be used to elucidate conformational state of subunits.

291-Pos

Investigation of the Mobile Regions of GM2 Activator Protein Using Continuous Wave and Pulsed Electron Paramagnetic Resonance and Fluorescence Spectroscopy

Jeff D. Carter, Gail E. Fanucci.
University of Florida, Gainesville, FL, USA.

GM2AP is an 18kDa protein that is involved in the catabolism of the ganglioside GM2. GM2AP is thought to bind GM2 in intralysosomal vesicles and present the oligosaccharide head group for hydrolytic cleavage. Mutations in GM2AP lead to an accumulation of GM2 in the lysosomes, causing the lysosomal storage diseases Tay Sachs or the AB variant of Sandhoff's disease. The crystal structure of GM2AP revealed the protein in five different crystal forms, with large differences in the diameter and area of the opening to the lipid binding cavity differences can be attributed to the flexibility of the mobile loops. Site directed spin-labeling combined with continuous wave and pulsed electron paramagnetic resonance methods has been used to investigate the intramolecular distances between the mobile loop regions of GM2AP. Distance profiles are obtained with and without the physiological ligand GM2 as other phospholipids.